

WEST Search History

DATE: Monday, February 07, 2005

Hide?	<u>Set</u> <u>Name</u>	<u>Query</u>	<u>Hit</u> <u>Count</u>
	<i>DB=USPT; PLUR=YES; OP=AND</i>		
<input type="checkbox"/>	L1	(\$toxin or botulin or botulinum or botox or dysport or botulism or neurotoxin or tetanus or tetox).clm.	3716
<input type="checkbox"/>	L2	L1 and (light and heavy).clm.	83
<input type="checkbox"/>	L3	l-chain.clm. and h-chain.clm. not antibod\$.clm.	1
	<i>DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND</i>		
<input type="checkbox"/>	L4	(recombinant or modified or altered or alteration or domains or portion or fragment or truncate or truncation).clm.	1510727
<input type="checkbox"/>	L5	(clostridial or clostridium or clostrid or botulinum or botulism or botulin or botox or btn or btx or rbotulin or neurotoxin).clm.	1552
<input type="checkbox"/>	L6	hybrid.clm.	17634
<input type="checkbox"/>	L7	(L6 or l4) and l5	596
<input type="checkbox"/>	L8	L7 not l2	585
<input type="checkbox"/>	L9	L8 and (botulinum or botulin or botulism or clostridial or clostridium or clostrid)	507
<input type="checkbox"/>	L10	L9 and binding	398
<input type="checkbox"/>	L11	L9 and receptor	268
<input type="checkbox"/>	L12	L11 and l10	255
<input type="checkbox"/>	L13	L12 and (channel\$ or pore\$ or translocation or translocate or hn or h-n)	179
<input type="checkbox"/>	L14	l13 and williams.in.	9
<input type="checkbox"/>	L15	allergan.asn.	2435
<input type="checkbox"/>	L16	L15 and williams.in.	26
	<i>DB=USPT; PLUR=YES; OP=AND</i>		
<input type="checkbox"/>	L17	US-6290960-B1.did.	1
<input type="checkbox"/>	L18	US-6365158-B1.did.	1
<input type="checkbox"/>	L19	US-5736139-A.did.	1
<input type="checkbox"/>	L20	US-6613329-B1.did.	1
<input type="checkbox"/>	L21	US-6573003-B2.did.	1
<input type="checkbox"/>	L22	US-6365158-B1.did.	1
<input type="checkbox"/>	L23	US-6290960-B1.did.	1
<input type="checkbox"/>	L24	US-5919665-A.did.	1

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		<i>DB=USPT; PLUR=YES; OP=AND</i>	
<input type="checkbox"/>	L1	(\$toxin or botulin or botulinum or botox or dysport or botulism or neurotoxin or tetanus or tetox).clm.	3716
<input type="checkbox"/>	L2	L1 and (light and heavy).clm.	83
<input type="checkbox"/>	L3	l-chain.clm. and h-chain.clm. not antibod\$.clm.	1

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		<i>DB=USPT; PLUR=YES; OP=AND</i>	
<input type="checkbox"/>	L1	5919665.pn.	1
<input type="checkbox"/>	L2	botulinum.clm. same toxin.clm.	121
<input type="checkbox"/>	L3	L2 and (truncat\$ or domain or domains or region or regions or portion or portions or chain or chains or fragment or fragments).clm.	47

END OF SEARCH HISTORY

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-
- ☐ 1. [6843998](#). 13 Apr 00; 18 Jan 05. Methods and compositions for the treatment of pancreatitis. Steward; Lance E., et al. 424/236.1; 424/197.11 424/198.1 424/247.1. A61K039/02.
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- ☐ 2. [6822076](#). 27 Aug 02; 23 Nov 04. Hybrid protein for inhibiting the degranulation of mastocytes and the use thereof. Bigalke; Hans, et al. 530/350; 424/192.1 435/7.1 530/300. C07K001/00.
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- ☐ 3. [6815540](#). 15 Jan 99; 09 Nov 04. Immunoglobulin superfamily domains and fragments with increased solubility. Pluckthun; Andreas, et al. 536/23.53; 435/320.1 435/325 435/326 530/387.3. C07H021/04.
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- ☐ 4. [6809184](#). 27 Sep 00; 26 Oct 04. Antibodies, including FV molecules, and immunoconjugates having high binding affinity for mesothelin and methods for their use. Pastan; Ira H., et al. 530/387.3; 424/130.1 424/183.1 530/388.85 530/391.3. A61K039/395.
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- ☐ 5. [6790938](#). 03 Dec 99; 14 Sep 04. Anti-GPIIb/IIIa recombinant antibodies. Berchtold; Peter, et al. 530/388.22; 424/130.1 530/387.1 530/388.85. C07K016/00.
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- ☐ 6. [6787517](#). 29 Dec 00; 07 Sep 04. Agent and methods for treating pain. Gil; Daniel W., et al. 514/1; 514/14 514/2. A01N061/00 A01N037/18 B61K031/00 B61K038/00 B61K038/28.
-
- ☐ 7. [6787153](#). 21 Dec 99; 07 Sep 04. Human monoclonal antibody specifically binding to surface antigen of cancer cell membrane. Hosokawa; Saiko, et al. 424/450; 424/133.1 424/134.1 424/138.1 424/142.1 424/155.1 424/174.1 530/387.1 530/387.7 530/388.15 530/388.8 530/389.7 530/391.1 530/391.7 530/865 530/866 530/867. A61K009/127 A61K039/395 A61K039/44 C07K016/30 C07K016/18.
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- ☐ 8. [6767733](#). 10 Oct 01; 27 Jul 04. Portable biosensor apparatus with controlled flow. Green; Larry R.. 435/288.5; 422/82.11 435/287.2 435/288.7 435/4 436/164. C12M001/34 C12Q001/00.
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- ☐ 9. [6765087](#). 19 Apr 99; 20 Jul 04. Immunoglobulins devoid of light chains. Casterman; Cecile, et al. 530/387.1; 530/387.3 530/388.1 530/391.1 530/391.5 530/391.7 530/391.9. C07K016/00 C07K017/00.
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- ☐ 10. [6752990](#). 31 Oct 01; 22 Jun 04. High affinity humanized anti-TAG-72 monoclonal antibodies. Anderson; W. H. Kerr, et al. 424/181.1; 424/178.1 435/188 530/388.85 530/391.7. A61K039/395.
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- ☐ 11. [6743896](#). 20 Sep 01; 01 Jun 04. Single-chain antigen-binding proteins capable of glycosylation, production and uses thereof. Filpula; David, et al. 530/387.3; 435/188 530/391.1 530/391.7. C07K016/00.
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- ☐ 12. [6737267](#). 22 Jan 01; 18 May 04. Non-peptidyl moiety-conjugated CD4-gamma2 and CD4-IgG2 immunoconjugates, and uses thereof. Maddon; Paul J., et al. 435/320.1; 424/1.53 424/179.1 530/388.35. C12N015/74 A61K051/00 C07K017/00.
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- ☐ 13. [6693176](#). 18 Jul 00; 17 Feb 04. Antitumor antibodies, proteins, and uses thereof. Rock; Kenneth L., et al. 530/388.75; 435/330 435/346 530/388.7 530/388.8. C07K016/18.
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- ☐ 14. 6652852. 28 Nov 00; 25 Nov 03. Chimeric antibody with specificity to human B cell surface antigen. Robinson; Randy R., et al. 424/133.1; 424/153.1 424/155.1 424/178.1 424/182.1 424/183.1 530/387.3 530/388.73 530/391.1 530/391.3 530/391.7. A61K039/395 C07K016/00.
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- ☐ 15. 6632928. 06 Dec 99; 14 Oct 03. Immunotoxins and methods of inducing immune tolerance. Neville; David M., et al. 530/388.75; 530/387.1 530/387.3 530/388.22 530/388.73. C07K016/00.
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- ☐ 16. 6631989. 17 Aug 01; 14 Oct 03. Non-invasive ocular assessment method and associated apparatus. Odom; James V., et al. 351/205; 351/246. A61B003/10.
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- ☐ 17. 6573241. 08 Jun 01; 03 Jun 03. Therapeutic agent for the suppression of snoring noises. Bigalke; Hans, et al. 514/12; 424/239.1 435/252.7 435/69.7 514/2 530/350 530/412 530/825. A61K038/16 A61P011/00.
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- ☐ 18. 6521230. 14 Mar 91; 18 Feb 03. CD25 binding molecules. Amlot; Peter Lloyd, et al. 424/154.1; 424/1.49 424/133.1 424/135.1 424/143.1 424/144.1 424/153.1 424/173.1 424/178.1 424/181.1 424/183.1 435/252.3 435/320.1 435/328 435/69.6 530/387.3 530/388.22 530/388.7 530/388.73 530/388.75 530/391.1 530/391.3 530/391.7 536/23.1 536/23.4 536/23.5 536/23.53. A61K039/395 C07K016/28 C12N015/13.
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- ☐ 19. 6512097. 20 May 99; 28 Jan 03. High affinity human antibodies to tumor antigens. Marks; James D., et al. 530/391.1; 530/387.3 530/387.7 530/388.1 530/388.15 530/388.2 530/388.22 530/388.24 530/388.8 530/388.85 530/391.3 530/391.7 530/391.9 530/395. C07K016/30 C07K016/46 C12P021/08.
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- ☐ 20. 6495137. 30 Oct 97; 17 Dec 02. Humanized anti-tag-72 monoclonal antibodies using human subgroup 4 kappa light chains. Mezes; Peter S., et al. 424/133.1; 424/1.49 424/1.53 424/138.1 424/156.1 424/178.1 435/7.23 530/387.3 530/388.85 530/391.3 530/391.5 530/391.7 530/866 530/867. A61K039/395 C07K016/30 G01N033/574.
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- ☐ 21. 6461824. 06 Jun 95; 08 Oct 02. Production of chimeric antibodies with specificity to human tumor antigens. Better; Marc D., et al. 435/7.23; 424/1.49 424/133.1 424/178.1 424/9.34 435/69.6 530/387.3 530/391.1 530/391.3 530/391.7. A61K039/395 C07K016/30 G01N033/574.
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- ☐ 22. 6461617. 23 Feb 99; 08 Oct 02. Recombinant toxin fragments. Shone; Clifford Charles, et al. 424/236.1; 424/157.1 424/164.1 424/167.1 424/178.1 424/179.1 424/184.1 424/234.1 424/235.1 424/239.1 424/247.1 435/252.33 435/69.1 435/69.7 435/70.1 435/71.1 435/71.2 530/300 530/350 530/825 536/23.4 536/23.7. A61K039/02 A61K039/38 A61K039/00 C12P021/06 C12P021/04.
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- ☐ 23. 6458354. 25 Sep 00; 01 Oct 02. Molecular complexes which modify immune responses. Schneck; Jonathan, et al. 424/134.1; 424/130.1 424/139.1 424/141.1 514/12 514/2 530/350 530/387.3 530/388.1. A61K039/395 C07K016/100 C12P021/08.
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- ☐ 24. 6451313. 07 Jun 95; 17 Sep 02. CD4-gamma2 and CD4-IGG2 chimeras. Maddon; Paul J., et al. 424/185.1; 424/1.49 424/1.69 424/134.1 424/184.1 424/192.1 435/328 435/358 435/361 435/365 435/69.1 435/69.7 530/350 530/387.3. A61K038/17 C07K014/705 C12N015/00.
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- ☐ 25. 6444209. 03 Nov 00; 03 Sep 02. Hybrid botulinal neurotoxins. Johnson; Eric A., et al. 424/194.1; 424/239.1 435/220 435/69.1 435/69.7 435/842 514/12 530/350 530/402 530/412 530/825 536/23.2 536/23.7. A61K039/385 A61K039/08 C12N009/52.
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- ☐ 26. 6395513. 22 Nov 99; 28 May 02. Clostridial toxin derivatives able to modify peripheral sensory afferent functions. Foster; Keith Alan, et al. 435/69.3; 435/69.1 435/69.7 530/350. C12N015/62 C12N015/09 C12P021/00 C07K019/00.
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- ☐ 27. 6383487. 06 Jun 95; 07 May 02. Methods of treatment using CD25 binding molecules. Amlot; Peter Lloyd, et al. 424/181.1; 424/1.49 424/130.1 424/133.1 424/135.1 424/141.1 424/143.1 424/144.1 424/153.1 424/173.1 424/178.1 424/183.1 530/387.1 530/387.3 530/388.1 530/388.2 530/388.22 530/388.7 530/388.73 530/388.75 530/391.1 530/391.3 530/391.7. A61K039/395 C07K016/28 C07K016/30.
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- ☐ 28. 6358733. 19 May 00; 19 Mar 02. Expression of heterologous multi-domain proteins in yeast. Motwani; Nalini, et al. 435/320.1; 435/69.1 536/23.1. C12N015/63 C07H021/04 C12P021/02.
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- ☐ 29. 6348581. 18 Feb 98; 19 Feb 02. High affinity humanized anti-TAG-72 monoclonal antibodies. Anderson; W. H. Kerr, et al. 530/388.85; 424/156.1 424/183.1 530/387.3 530/388.8 530/391.7. A61K039/395.
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- ☐ 30. 6329507. 21 Aug 92; 11 Dec 01. Dimer and multimer forms of single chain polypeptides. Mezes; Peter S., et al. 530/387.3; 435/320.1 536/23.1. C12P021/08.
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- ☐ 31. 6323322. 30 Apr 98; 27 Nov 01. Single-chain antigen-binding proteins capable of glycosylation, production and uses thereof. Filpula; David, et al. 530/387.3; 530/391.3. C07K016/00.
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- ☐ 32. 6309636. 14 Sep 95; 30 Oct 01. Recombinant peptides derived from the Mc3 anti-BA46 antibody, methods of use thereof, and methods of humanizing antibody peptides. do Couto; Fernando J. R., et al. 424/133.1; 424/134.1 424/138.1 424/141.1 424/152.1 424/172.1 424/174.1 424/178.1 424/183.1 435/7.1 530/350 530/388.1. A61K033/395 C07K001/00 C07K016/00 G01N033/53.
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- ☐ 33. 6287562. 08 Jan 99; 11 Sep 01. Methods of inhibiting the growth of cells bearing Lewis Y antigens using B1, B3, or B5 targeted immunoconjugates. Pastan; Ira, et al. 424/183.1; 424/156.1 424/178.1 424/181.1 530/387.3 530/388.8 530/388.85 530/391.1 530/391.7. A61K039/395.
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- ☐ 34. 6268411. 10 Sep 98; 31 Jul 01. Use of multivalent chimeric peptide-loaded, MHC/ig molecules to detect, activate or suppress antigen-specific T cell-dependent immune responses. Schneck; Jonathan, et al. 524/12;. C07K016/100 C12P021/08.
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- ☐ 35. 6214602. 28 Aug 98; 10 Apr 01. Host cells for expression of clostridial toxins and proteins. Zdanovsky; Alexey G.. 435/252.3; 435/325. C12N001/20 C12N005/10.
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- ☐ 36. 6207804. 18 Dec 95; 27 Mar 01. Genetically engineered antibody analogues and fusion proteins thereof. Huston; James S., et al. 530/387.3; 530/387.9 530/388.1. C12P021/08.
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- ☐ 37. 6207153. 22 May 97; 27 Mar 01. Antigen binding fragments that specifically detect cancer cells, nucleotides encoding the fragments, and use thereof for the prophylaxis and detection of cancers. Dan; Michael D., et al. 424/138.1; 424/141.1 424/142.1 424/155.1 530/387.7 530/388.8 530/391.1 530/391.3 530/391.7. A61K039/395.
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- ☐ 38. 6203794. 01 May 97; 20 Mar 01. Modification of clostridial toxins for use as transport proteins. Dolly; James Oliver, et al. 424/184.1; 424/164.1 424/167.1 424/178.1 424/179.1 424/183.1 424/234.1 424/235.1 424/236.1 424/239.1 424/247.1 424/832 530/300 530/350. A61K039/395
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A61K039/02 A61K038/00 C07K014/00.

☐ 39. 6187287. 03 Aug 98; 13 Feb 01. Immunoconjugates and humanized antibodies specific for B-cell lymphoma and leukemia cells. Leung; Shui-on, et al. 424/9.1; 424/134.1 424/135.1 424/138.1 424/141.1 435/320.1 435/69.7 530/387.3 530/388.8 530/391.7 536/23.4. A61K049/00 A61K039/395 C12P021/08 C12N015/00 C07K016/30.

☐ 40. 6147203. 05 Jan 98; 14 Nov 00. Recombinant disulfide-stabilized polypeptide fragments having binding specificity. Pastan; Ira H., et al. 536/23.53; 536/23.1. C07H021/02 C07H021/04.

☐ 41. 6139869. 25 May 95; 31 Oct 00. Human monoclonal antibody specifically binding to surface antigen of cancer cell membrane. Hosokawa; Saiko, et al. 424/450; 424/138.1 424/142.1 424/155.1 424/174.1 424/812 435/330 435/344 435/372.2 530/387.7 530/388.15 530/388.8 530/391.1 530/809. A61K009/127 A61K009/133 A61K039/395 C07K016/30.

☐ 42. 6130364. 29 Mar 95; 10 Oct 00. Production of antibodies using Cre-mediated site-specific recombination. Jakobovits; Aya, et al. 800/6; 435/326 435/463 435/69.1 800/13 800/18 800/4. C12N015/85 C12N015/00 A01K067/00.

☐ 43. 6120767. 12 Feb 98; 19 Sep 00. Chimeric antibody with specificity to human B cell surface antigen. Robinson; Randy R., et al. 424/133.1; 424/1.49 424/153.1 424/155.1 424/178.1 424/182.1 424/183.1 530/387.3 530/388.73 530/391.1 530/391.3 530/391.7. A61K039/395 C07K016/30.

☐ 44. 6099842. 03 Dec 90; 08 Aug 00. Recombinant immunotoxin composed of a single chain antibody reacting with the human transferrin receptor and diphtheria toxin. Pastan; Ira, et al. 424/183.1; 424/178.1 530/391.7. A61K039/40 A61K039/42 A61K039/44 A61K039/395.

☐ 45. 6074644. 17 Sep 99; 13 Jun 00. Nucleic acids encoding immunotoxins containing a disulfide-stabilized antibody fragment replacing half or more of domain IB of pseudomonas exotoxin, and methods of use of the encoded immunotoxins. Pastan; Ira, et al. 424/178.1; 424/236.1 530/387.3 530/387.7 536/23.1. A61K039/395 C07K016/00.

☐ 46. 6030796. 07 Jun 95; 29 Feb 00. Monoclonal antibody to a human MDR1 multidrug resistance gene product, and uses. Metchetner; Eugene, et al. 435/7.23; 424/133.1 424/143.1 424/155.1 435/328 435/330 435/334 530/387.3 536/23.53. G01N033/574 A61K039/395 C07H021/04 C12P021/08.

☐ 47. 6015884. 28 Mar 97; 18 Jan 00. Soluble divalent and multivalent heterodimeric analogs of proteins. Schneck; Jonathan, et al. 530/387.3; 530/388.1. C07K016/00 C12P021/08.

☐ 48. 5989545. 12 Jan 98; 23 Nov 99. Clostridial toxin derivatives able to modify peripheral sensory afferent functions. Foster; Keith Alan, et al. 424/183.1; 424/832 424/94.67 435/220 435/69.1 435/69.7 514/2 530/350 530/388.22 530/391.7 530/402. A61K038/16 C07K014/33 C07K019/00 C12N015/62.

☐ 49. 5980896. 14 Jun 93; 09 Nov 99. Antibodies reactive with human carcinomas. Hellstrom; Ingegerd, et al. 424/183.1; 424/134.1 424/135.1 424/136.1 424/138.1 424/141.1 424/155.1 424/178.1 424/181.1 530/387.3 530/387.5 530/387.7 530/391.7. A61K038/395 C07K016/30 C07K019/00 C12N005/12.

☐ 50. 5980895. 21 Aug 97; 09 Nov 99. Immunotoxin containing a disulfide-stabilized antibody fragment joined to a Pseudomonas exotoxin that does not require proteolytic activation. Pastan; Ira, et al. 424/178.1; 424/236.1 530/387.3 530/387.7. A61K039/395 C07K016/00.

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L1 and (light and heavy).clm.	83

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Search Results - Record(s) 1 through 9 of 9 returned.

- ☐ 1. 20040253673. 05 Dec 03. 16 Dec 04. Recombinant botulinum toxins with a soluble C-terminal portion. Williams, James A.. 435/69.1; 435/252.33 435/254.2 435/320.1 435/348 530/350 536/23.7 C07K014/33 C07H021/04 C12N001/21.
- ☐ 2. 20040235118. 04 Dec 03. 25 Nov 04. Portions of soluble recombinant botulinum toxins. Williams, James A.. 435/69.7; C12P021/04.
- ☐ 3. 20040219637. 05 Dec 03. 04 Nov 04. Soluble recombinant botulinum toxins having a C-terminal portion of a heavy chain, a N-terminal portion of a heavy chain and a light chain. Williams, James A.. 435/69.3; 435/252.33 435/254.2 435/320.1 435/348 C12P021/02 C12N001/18 C12N005/06.
- ☐ 4. 20040142455. 05 Dec 03. 22 Jul 04. Recombinant botulinum toxins having a soluble C-terminal portion of a heavy chain, an N-terminal portion of a heavy chain and a light chain. Williams, James A.. 435/252.33; 435/254.2 435/320.1 435/348 435/69.3 530/350 536/23.7 C12P021/02 C12N001/21 C12N001/18 C12N005/06.
- ☐ 5. 20040115215. 05 Dec 03. 17 Jun 04. Recombinant botulinum toxins with a soluble C-terminal portion, an N-terminal portion and a light chain. Williams, James A.. 424/184.1; A61K039/395 A61K039/00 A61K039/38.
- ☐ 6. 20030219457. 15 Oct 02. 27 Nov 03. Soluble recombinant botulinum toxins. Williams, James A.. 424/199.1; 424/186.1 424/234.1 435/6 C12Q001/68 A61K039/12 A61K039/02.
- ☐ 7. 20030215468. 30 Jan 03. 20 Nov 03. Soluble recombinant botulinum toxin proteins. Williams, James A., et al. 424/239.1; 435/252.3 435/70.21 530/388.4 A61K039/08 C12P021/04 C12N001/21 C07K016/12.
- ☐ 8. 20030118547. 14 Nov 02. 26 Jun 03. Composition for intestinal delivery. Vandenberg, Grant William. 424/85.4; 424/130.1 424/85.2 424/93.2 514/169 514/2 514/54 514/560 A61K048/00 A61K038/21 A61K031/715 A61K038/24 A61K038/20 A61K031/573 A61K031/20 A61K031/56 A61K039/395.
- ☐ 9. 20030108597. 13 Aug 02. 12 Jun 03. Application of lipid vehicles and use for drug delivery. Chancellor, Michael B., et al. 424/450; 424/143.1 424/239.1 424/760 514/44 514/625 A61K048/00 A61K039/395 A61K009/127 A61K035/78 A61K031/16 A61K039/08.

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L13 and williams.in.	9

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DATE: Monday, February 07, 2005

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<input type="checkbox"/>	L3	l-chain.clm. and h-chain.clm. not antibod\$.clm.	1
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<input type="checkbox"/>	L5	(clostridial or clostridium or clostrid or botulinum or botulism or botulin or botox or btn or btx or rbotulin or neurotoxin).clm.	1552
<input type="checkbox"/>	L6	hybrid.clm.	17634
<input type="checkbox"/>	L7	(L6 or l4) and l5	596
<input type="checkbox"/>	L8	L7 not l2	585
<input type="checkbox"/>	L9	L8 and (botulinum or botulin or botulism or clostridial or clostridium or clostrid)	507
<input type="checkbox"/>	L10	L9 and binding	398
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<input type="checkbox"/>	L12	L11 and l10	255
<input type="checkbox"/>	L13	L12 and (channel\$ or pore\$ or translocation or translocate or hn or h-n)	179
<input type="checkbox"/>	L14	l13 and williams.in.	9
<input type="checkbox"/>	L15	allergan.asn.	2435
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<input type="checkbox"/>	L18	US-6365158-B1.did.	1
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<input type="checkbox"/>	L24	US-5919665-A.did.	1

END OF SEARCH HISTORY

DOCUMENT-IDENTIFIER: US 4664911 A

TITLE: Immunotoxin conjugates employing toxin B chain moieties

CLAIMS:

1. A cytotoxic composition comprising, in combination:

a first conjugate comprising a specific binding agent covalently coupled to toxin A chain or toxin B chain moiety, together with

a second conjugate comprising a binding agent having an affinity for a cell surface structure of a target cell or for the binding agent of the first conjugate, said binding agent being covalently coupled to toxin A chain or toxin B chain moiety, the first conjugate and second conjugate each having a different toxin chain.

3. The composition according to claim 1 wherein each binding agent is a F(ab') antibody fragment.

4. The composition according to claim 1 wherein the toxin A chain and toxin B chain are the respective A and B chain moieties derived from the toxins ricin, abrin, modeccin, viscumin, cholera, E. coli heat-labile, pertussis, tetanus, botulinum, Pseudomonas, shigella or diphtheria.

5. The composition according to claim 1 wherein the toxin A chain moiety is ricin A chain and wherein the toxin B chain moiety is ricin B chain.

11. A method for potentiating the cytotoxicity of toxin A chain containing conjugates effective to selectively delete target cells from a population of cells, the method comprising:

contacting the population of cells with a first conjugate comprising

a binding agent having an affinity for an antigenic determinate of the target cell surface, the binding agent being covalently coupled to a toxin A chain, and

a second conjugate comprising

a binding agent having affinity for an antigenic determinant of the target cell surface or for an antigenic determinant of the binding agent of the first conjugate, the binding agent being covalently coupled to toxin B chain,

the amount of the combination of the first conjugate and the second conjugate being an amount effective to selectively delete a significant portion of target cells from a population of cells.

13. The method according to claim 11 wherein at least one binding agent is a F(ab') antibody fragment.

14. The method according to claim 11 wherein the toxin A chain moiety and toxin B chain moiety are the respective A and B chain moieties derived from the toxins ricin, abrin, modeccin, viscumin, cholera, E. coli heat-labile, pertussis, tetanus, botulinum, Pseudomonas, shigella or diphtheria.

15. The method according to claim 11 wherein the toxin A chain moiety is ricin A chain and the toxin B chain moiety is ricin B chain.

United States Patent [19]

Uhr et al.

[11] Patent Number: 4,664,911

[45] Date of Patent: May 12, 1987

[54] IMMUNOTOXIN CONJUGATES EMPLOYING TOXIN B CHAIN MOIETIES

[75] Inventors: Jonathan W. Uhr; Ellen S. Vitetta,
both of Dallas, Tex.

[73] Assignee: Board of Regents, University of Texas
System, Austin, Tex.

[21] Appl. No.: 506,540

[22] Filed: Jun. 21, 1983

[51] Int. Cl.⁴ A61K 39/00; G01N 33/563;
G01N 33/53; G01N 33/554

[52] U.S. Cl. 424/85; 424/88;
436/512; 436/519; 436/547; 436/813; 436/879;
530/387; 530/388

[58] Field of Search 260/112 B, 112 R;
424/85, 88; 436/512, 519, 547, 813; 530/387,
388

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Primary Examiner—Esther M. Kepplinger

Assistant Examiner—Jeremy Jay

Attorney, Agent, or Firm—Arnold, White & Durkee

[57]

ABSTRACT

Compositions and methods for potentiating the cytotoxic activity of immunotoxin conjugates are provided. The compositions of the present invention include a selective binding agent such as an antibody coupled to a toxin B chain moiety such as ricin B chain.

19 Claims, No Drawings

US-PAT-NO: 5919665

DOCUMENT-IDENTIFIER: US 5919665 A

TITLE: Vaccine for clostridium botulinum neurotoxin

DATE-ISSUED: July 6, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Williams; James A.	Madison	WI		

US-CL-CURRENT: 435/71.1, 435/252.3, 435/320.1, 530/350, 530/825, 536/23.4

CLAIMS:

I claim:

1. A soluble fusion protein comprising a non-toxin protein sequence and a portion of the Clostridium botulinum type A toxin, said portion of the Clostridium botulinum type A toxin comprising a portion of the sequence of SEQ ID NO:28.
2. The fusion protein of claim 1, wherein said portion of the Clostridium botulinum type A toxin sequence comprises SEQ ID NO:23.
3. The fusion protein of claim 1, wherein said non-toxin protein sequence comprises a poly-histidine tract.
4. The fusion protein of claim 3, which comprises SEQ ID NO:26.
5. The fusion protein of claim 1, wherein said fusion protein is substantially endotoxin-free.
6. A host cell containing a recombinant expression vector, said vector encoding encoding a protein comprising at least a portion of a Clostridium botulinum type A toxin protein sequence of SEQ ID NO:28, and wherein said host cell is capable of expressing said protein as a soluble protein in said host cell at a level greater than or equal to 0.75% of the total cellular protein.
7. The host cell of claim 6, wherein said portion of a toxin comprises SEQ ID NO:23.
8. The host cell of claim 6, wherein said fusion protein comprises SEQ ID NO:26.
9. The host cell of claim 6, wherein said host cell is capable of expressing said protein in said host cell at a level greater than or equal to 20% of the total cellular protein.
10. A soluble fusion protein, comprising at least a portion of Clostridium botulinum C fragment linked to a poly-histidine tag.

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US005919665A

United States Patent [19]
Williams

[11] **Patent Number:** **5,919,665**
 [45] **Date of Patent:** **Jul. 6, 1999**

[54] **VACCINE FOR CLOSTRIDIUM BOTULINUM NEUROTOXIN**

[75] **Inventor:** James A. Williams, Madison, Wis.

[73] **Assignee:** Ophidian Pharmaceuticals, Inc., Madison, Wis.

[21] **Appl. No.:** 08/405,496

[22] **Filed:** Mar. 16, 1995

Related U.S. Application Data

[63] Continuation-in-part of application No. 08/329,154, Oct. 25, 1994, abandoned, which is a continuation-in-part of application No. 08/161,907, Dec. 2, 1993, Pat. No. 5,601,823, which is a continuation-in-part of application No. 08/985,321, Dec. 4, 1992, which is a continuation-in-part of application No. 07/429,791, Oct. 31, 1989, Pat. No. 5,196,193.

[51] **Int. Cl.⁶** C07K 19/00; C12N 1/20; C12P 1/00

[52] **U.S. Cl.** 435/71.1; 435/252.3; 435/320.1; 530/825; 530/350; 536/23.4

[58] **Field of Search** 435/252.3, 320.1, 435/71.1; 536/23.4; 530/825, 350

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Primary Examiner—Frank C. Eisenschenk

Assistant Examiner—Evelyn Rabin

Attorney, Agent, or Firm—Medlen & Carroll, LLP

[57]

ABSTRACT

The present invention includes recombinant proteins derived from *Clostridium botulinum* toxins. In particular, soluble recombinant *Clostridium botulinum* type A toxin proteins are provided. Methods which allow for the isolation of recombinant proteins free of significant endotoxin contamination are provided. The soluble, endotoxin-free recombinant proteins are used as immunogens for the production of vaccines and antitoxins. These vaccines and antitoxins are useful in the treatment of humans and other animals at risk of intoxication with clostridial toxin.

10 Claims, 29 Drawing Sheets

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DERWENT-ACC-NO: 1998-230234

DERWENT-WEEK: 200482

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TITLE: Host cell containing recombinant expression vector encoding Clostridium botulinum type B or E toxin - useful to treat humans and other animals at risk of intoxication with clostridial toxin

INVENTOR: THALLEY, B S; WILLIAMS, J A

PATENT-ASSIGNEE: OPHIDIAN PHARM INC (OPHIN), ALLERGAN BOTOX LTD (ALLR),
ALLERGAN INC (ALLR), ALLERGAN SALES INC (ALLR)

PRIORITY-DATA: 1996US-0704159 (August 28, 1996), 1995US-0405496 (March 16, 1995), 2003US-0354774 (January 30, 2003), 2002US-0271012 (October 15, 2002), 2003US-0729122 (December 5, 2003), 2003US-0729039 (December 5, 2003), 2003US-0729527 (December 5, 2003), 2003US-0727898 (December 4, 2003), 2003US-0728696 (December 5, 2003)

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PATENT-FAMILY:

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DESIGNATED-STATES: AU CA JP AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
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US20040253673A1	October 15, 2002	2002US-0271012	Div ex
US20040253673A1	December 5, 2003	2003US-0728696	
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EP 1105153A1		WO 9808540	Based on
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US20040235118A1	January 30, 2003	2003US-0354774	Div ex
US20040235118A1	December 4, 2003	2003US-0727898	
US20040235118A1		US 5919665	CIP of

INT-CL (IPC): A61 K 38/08; A61 K 39/00; A61 K 39/02; A61 K 39/08; A61 K 39/12; A61 K 39/38; A61 K 39/395; C07 H 21/04; C07 K 14/33; C07 K 16/00; C07 K 16/12; C12 N 1/18; C12 N 1/21; C12 N 5/06; C12 N 15/00; C12 N 15/09; C12 N 15/63; C12 N 15/70; C12 N 15/74; C12 P 21/02; C12 P 21/04; C12 P 21/06; C12 P 21/08; C12 Q 1/68

RELATED-ACC-NO: 1994-217494;1994-271898 ;1994-341765 ;1996-230603

ABSTRACTED-PUB-NO: WO 9808540A

BASIC-ABSTRACT:

Host cell, containing a recombinant expression vector, which encodes a protein comprising at least a portion of a Clostridium botulinum type B or E toxin, is claimed. Also claimed are: (1) a host cell containing a recombinant expression vector, which encodes a fusion protein comprising a non-toxin protein sequence, preferably comprising a poly-histidine tract, and at least a portion, preferably comprising the receptor binding domain, of a C. botulinum type B or E toxin; and (2) a vaccine, preferably endotoxin free, comprising the fusion protein of (1), and preferably further comprising a fusion protein comprising a non-toxin protein sequence and at least a portion of C. botulinum type A toxin.

USE - An antigen comprising the fusion protein can be used to generate a novel antibody (Ab) directed against a C. botulinum toxin (claimed). The vaccine and the Ab can be used to treat humans and other animals at risk of intoxication with clostridial toxin, while the Ab or the protein can also be used for the detection of bacterial toxins.

ABSTRACTED-PUB-NO: WO 9808540A

EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/40

DERWENT-CLASS: B04 D16

CPI-CODES: B04-E08; B04-G01; B04-N0300E; B12-K04A4; B14-A01; B14-S11B; D05-H07; D05-H11; D05-H14A1; D05-H17C;

Entry 25 of 83

File: USPT

Sep 3, 2002

US-PAT-NO: 6444209

DOCUMENT-IDENTIFIER: US 6444209 B1

**** See image for Certificate of Correction ****

TITLE: Hybrid botulinal neurotoxins

DATE-ISSUED: September 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Johnson; Eric A.	Madison	WI		
Goodnough; Michael C.	Stoughton	WI		
Bradshaw; Marite	Madison	WI		
Tepp; William H.	Stoughton	WI		

US-CL-CURRENT: 424/194.1; 424/239.1, 435/220, 435/69.1, 435/69.7, 435/842, 514/12, 530/350, 530/402, 530/412, 530/825, 536/23.2, 536/23.7

CLAIMS:

We claim:

1. A hybrid botulinal neurotoxin comprising: (a) a botulinal neurotoxin light chain; and (b) a botulinal neurotoxin heavy chain, wherein the light chain and heavy chain are not of the same serotype and wherein the light and heavy chains are linked by a reducible, disulfide homobifunctional linker and wherein the specific toxicity of the neurotoxin is at least 10⁶ LD₅₀ /mg protein in vivo.
2. The neurotoxin of claim 1 wherein the heavy chain or light chain is isolated from a native botulinal neurotoxin molecule.
3. The neurotoxin of claim 1 wherein the heavy chain or light chain is obtained from a recombinant gene construct.
4. The neurotoxin of claim 1 wherein the entire hybrid neurotoxin is obtained from a recombinant gene construct.
5. A pharmaceutical composition comprising the neurotoxin of claim 1.



US006395513B1

(12) **United States Patent**
Foster et al.

(10) Patent No.: **US 6,395,513 B1**
(45) Date of Patent: ***May 28, 2002**

(54) **CLOSTRIDIAL TOXIN DERIVATIVES ABLE TO MODIFY PERIPHERAL SENSORY AFFERENT FUNCTIONS**

(75) Inventors: **Keith Alan Foster**, Wiltshire; **Michael John Duggan**, London; **Clifford Charles Shone**, Wiltshire, all of (GB)

(73) Assignees: **The Speywood Laboratory, Ltd.**, London; **Microbiological Research Authority**, Wiltshire, both of (GB)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/447,356**

(22) Filed: **Nov. 22, 1999**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/945,037, filed as application No. PCT/GB96/00916 on Apr. 16, 1996, now Pat. No. 5,989,545.

(30) **Foreign Application Priority Data**

Apr. 21, 1995 (GB) 9508204

(51) Int. Cl.⁷ **C12N 15/62; C12N 15/09; C12P 21/00; C07K 19/00**

(52) U.S. Cl. **435/69.3; 435/69.1; 435/69.7; 530/350**

(58) Field of Search **435/69.1, 69.3, 435/69.7; 530/350**

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Primary Examiner—Mary E. Mosher

(74) *Attorney, Agent, or Firm*—Foley & Lardner

(57) **ABSTRACT**

The invention relates to an agent specific for peripheral sensory afferents. The agent may inhibit the transmission of signals between a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent. The agent may be used in or as a pharmaceutical for the treatment of pain, particularly chronic pain.

7 Claims, 4 Drawing Sheets

-continued

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What is claimed is:

1. A method for preparing an agent in the form of a fusion protein, which agent binds to a peripheral sensory afferent, the agent comprising a Targeting Moiety (TM) coupled to a modified clostridial neurotoxin in which the TM comprises a ligand to a cell-surface binding site present on a primary sensory afferent and is capable of functionally interacting with the binding site causing a physical association between the agent and the surface of the primary sensory afferent,

wherein the heavy chain (H-chain) of the clostridial neurotoxin is removed or modified to remove or reduce its native binding affinity for motor neurons, and the light chain (L-chain) of the clostridial neurotoxin or a fragment thereof retains a protease activity specific for components of the neurosecretory machinery, the TM and the modified H-chain, if present, forming a molecule which introduces the L-chain or fragment thereof into the cytosol of a primary sensory afferent, thereby inhibiting the transmission of signals between

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a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent; said method comprising expressing in a host organism a genetic construct which codes for the agent.

2. A method according to claim 1 which further comprises constructing the genetic construct and transforming the host with said construct.

3. A method according to claim 1, wherein the genetic construct includes a sequence encoding a spacer molecule by which the modified clostridial neurotoxin, or a fragment thereof, is coupled to the TM.

4. A method according to claim 3, wherein the spacer molecule is selected from the group consisting of (SEQ ID NO: 11) PPPIEGR, collagen-like spacer, and trypsin-sensitive diphtheria toxin peptide.

5. A method according to claim 1, wherein the nucleic acid sequence of the genetic construct is modified in accordance with the codon bias of the host cell.

6. A method according to claim 1, wherein the genetic construct incorporates a nucleic acid sequence encoding an affinity tag to facilitate purification of the assembled toxin.

7. An agent which binds to a peripheral sensory afferent and has been obtained in the form of a fusion protein by the method according to claim 1, said agent comprising

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a Targeting Moiety (TM) coupled to a modified clostridial neurotoxin in which the TM comprises a ligand to a cell-surface binding site present on a primary sensory afferent and is capable of functionally interacting with the binding site causing a physical association between the agent and the surface of the primary sensory afferent,

wherein the heavy chain (H-chain) of the clostridial neurotoxin is removed or modified to remove or reduce its native binding affinity for motor neurons,

and the light chain (L-chain) of the clostridial neurotoxin or a fragment thereof retains a protease activity specific for components of the neurosecretory machinery,

the TM and the modified H-chain, if present, forming a molecule which introduces the L-chain or fragment thereof into the cytosol of a primary sensory afferent, thereby inhibiting the transmission of signals between a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent.

* * * * *

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L2: Entry 35 of 83

File: USPT

Apr 10, 2001

US-PAT-NO: 6214602

DOCUMENT-IDENTIFIER: US 6214602 B1

TITLE: Host cells for expression of clostridial toxins and proteins

DATE-ISSUED: April 10, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zdanovsky; Alexey G.	Madison	WI		

US-CL-CURRENT: [435/252.3](#); [435/325](#)

CLAIMS:

What is claimed is:

1. A host cell containing a recombinant expression vector, said vector encoding encoding transfer RNAs that recognize ATA, AGA, and CTA codons, and wherein said recombinant expression vector is selected from the group consisting of pACYC-RL5, pACYC-L10, pACYC-IRL10, and pACYC-IleArgLeu17.
2. The host cell of claim 1, wherein said host cell is capable of expressing at at least fragments of at least one clostridial protein.
3. The host cell of claim 2, wherein said clostridial proteins are selected from the group consisting of light chains of botulinal neurotoxins, heavy chains of botulinal neurotoxins, botulinal C3 protein, clostridial iota toxin Ia protein, and light and heavy chains of tetanus toxin .
4. The host cell of claim 2, wherein said clostridial protein is expressed at a level such that the clostridial protein ranges from 6 to 35 percent of the total cell protein.
5. The host cell of claim 2, wherein said clostridial protein is expressed at a level such that the clostridial protein ranges from 10 to 25 percent of the total cell protein.
6. The recombinant expression vector of claim 2, wherein said vector further comprises an affinity tag.

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US006214602B1

(12) **United States Patent**
Zdanovsky(10) Patent No.: **US 6,214,602 B1**
(45) Date of Patent: **Apr. 10, 2001**(54) **HOST CELLS FOR EXPRESSION OF CLOSTRIDIAL TOXINS AND PROTEINS**(75) Inventor: **Alexey G. Zdanovsky, Madison, WI (US)**(73) Assignee: **Promega Corporation, Madison, WI (US)**

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/143,634**(22) Filed: **Aug. 28, 1998**(51) Int. Cl.⁷ **C12N 1/20; C12N 5/10**(52) U.S. Cl. **435/252.3; 435/325**(58) Field of Search **435/320.1, 252.3, 435/254.2, 252.33, 325; 536/23.1, 24.3**(56) **References Cited****U.S. PATENT DOCUMENTS**4,683,195 7/1987 Mullis et al. .
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4,965,188 10/1990 Mullis et al. .**FOREIGN PATENT DOCUMENTS**

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(List continued on next page.)

Primary Examiner—Michael Pak
 Assistant Examiner—Sharon L. Turner

(57) **ABSTRACT**

The present invention is directed to methods and compositions useful in the overproduction of Clostridium toxins and proteins by hosts such as *E. coli*. These proteins and toxins find use in various medical and veterinary applications, including vaccine production, and cosmetic dermatology, as well as treatment of neurological and other diseases and conditions.

6 Claims, 8 Drawing Sheets

L2: Entry 48 of 83

File: USPT

Nov 23, 1999

DOCUMENT-IDENTIFIER: US 5989545 A

TITLE: Clostridial toxin derivatives able to modify peripheral sensory afferent functions

CLAIMS:

1. A non-cytotoxic agent which binds to a peripheral sensory afferent which comprises a Targeting Moiety (TM) coupled to a modified clostridial neurotoxin in which the TM comprises a ligand to a cell-surface binding site present on a primary sensory afferent and is capable of functionally interacting with a binding site causing a physical association between the agent and the surface of a primary sensory afferent; and the heavy chain (H-chain) of the clostridial neurotoxin is removed or modified by chemical derivitisation, mutation or proteolysis to reduce or remove its native binding affinity for motor neurons; and the light chain (L-chain) of the clostridial neurotoxin or a fragment thereof retains a protease activity specific for components of the neurosecretory machinery; the TM and the modified H-chain, if present, forming a molecule which introduces the L-chain or fragment thereof into the cytosol of a primary sensory afferent, thereby inhibiting the transmission of signals between a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent.
2. An agent according to claim 1 which comprises a Targeting Moiety (TM) coupled to a clostridial neurotoxin in which the H.sub.C part of the H-chain is removed or modified.
3. An agent according to claim 1 in which the modified H-chain is the H.sub.N- fragment of a clostridial neurotoxin.
4. An agent according to claim 1 in which the clostridial neurotoxin component is obtained from botulinum neurotoxin.
5. An agent according to claim 4 in which the clostridial neurotoxin component is obtained from botulinum neurotoxin selected from the group consisting of botulinum neurotoxin type A, botulinum neurotoxin type B, and botulinum neurotoxin type C.
6. An agent according to claim 5 which is formed by the coupling of a TM to the LH.sub.N fragment of botulinum neurotoxin type A.
7. An agent according to claim 5 which is formed by the coupling of a TM to the LH.sub.N fragment of botulinum neurotoxin type B.
8. An agent according to claim 5 which is formed by the coupling of a TM to the LH.sub.N fragment of botulinum neurotoxin type C1.
9. An agent according to claim 1 in which the H-chain is obtained from a different clostridial neurotoxin than that from which the L-chain is obtained.
10. An agent according to claim 9 in which the H-chain is obtained from botulinum neurotoxin type A and the L-chain from botulinum neurotoxin type B.
11. An agent according to claim 10 which is composed of a TM linked to the H.sub.N fragment of

botulinum neurotoxin type A and the L-chain of botulinum neurotoxin type B.

24. An agent according to claim 23 which comprises nerve growth factor linked to the LH.sub.N fragment of botulinum neurotoxin type A.

26. An agent according to claim 1 in which the TM is linked to the clostridial neurotoxin-derived component by a direct covalent linkage.

27. An agent according to claim 1 in which the TM is linked to the clostridial neurotoxin-derived component by a covalent linkage which includes one or more spacer regions.

37. A method for obtaining an agent according to claim 1, which comprises constructing a genetic construct which codes for a modified clostridial neurotoxin or a fragment of a clostridial neurotoxin, incorporating said construct into a host organism, expressing the construct to produce the modified clostridial neurotoxin or fragment of a clostridial neurotoxin, and covalently attaching said clostridial neurotoxin or fragment thereof to a TM.



US005989545A

United States Patent [19]

Foster et al.

[11] **Patent Number:** **5,989,545**[45] **Date of Patent:** **Nov. 23, 1999**

[54] **CLOSTRIDIAL TOXIN DERIVATIVES ABLE TO MODIFY PERIPHERAL SENSORY AFFERENT FUNCTIONS**

[75] **Inventors:** Keith Alan Foster, Wiltshire; Michael John Duggan, London; Clifford Charles Shone, Wiltshire, all of United Kingdom

[73] **Assignees:** The Speywood Laboratory Ltd., London; Microbiological Research Authority, Wiltshire, both of United Kingdom

[21] **Appl. No.:** **08/945,037**

[22] **PCT Filed:** **Apr. 16, 1996**

[86] **PCT No.:** **PCT/GB96/00916**

§ 371 Date: **Jan. 12, 1998**

§ 102(e) Date: **Jan. 12, 1998**

[87] **PCT Pub. No.:** **WO96/33273**

PCT Pub. Date: **Oct. 24, 1996**

[30] **Foreign Application Priority Data**

Apr. 21, 1995 [GB] United Kingdom 9508204

[51] **Int. Cl.⁶** **A61K 38/16; C07K 14/33; C07K 19/00; C12N 15/62**

[52] **U.S. Cl.** **424/183.1; 424/94.67; 424/832; 514/2; 530/388.22; 530/391.7; 530/350; 530/402; 435/69.1; 435/69.7; 435/220**

[58] **Field of Search** 424/832, 94.67, 424/183.1; 514/2; 530/388.22, 391.7, 350, 402; 435/69.1, 69.7, 220

[56] **References Cited**

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Primary Examiner—Mary E. Mosher

Attorney, Agent, or Firm—Foley & Lardner

[57] **ABSTRACT**

The invention relates to an agent specific for peripheral sensory afferents. The agent may inhibit the transmission of signals between a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent. The agent may be used in or as a pharmaceutical for the treatment of pain, particularly chronic pain.

43 Claims, 4 Drawing Sheets



US006787517B1

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extension
Hec: 7(12) **United States Patent**
Gil et al.(10) **Patent No.:** **US 6,787,517 B1**(45) **Date of Patent:** **Sep. 7, 2004**(54) **AGENT AND METHODS FOR TREATING PAIN**WO WO 96/01813 * 1/1996
WO WO96/33273 10/1996
WO WO01/78702 10/2001(75) Inventors: **Daniel W. Gil**, Corona Del Mar, CA (US); **Kei R. Aoki**, Coto de Caza, CA (US)**OTHER PUBLICATIONS**

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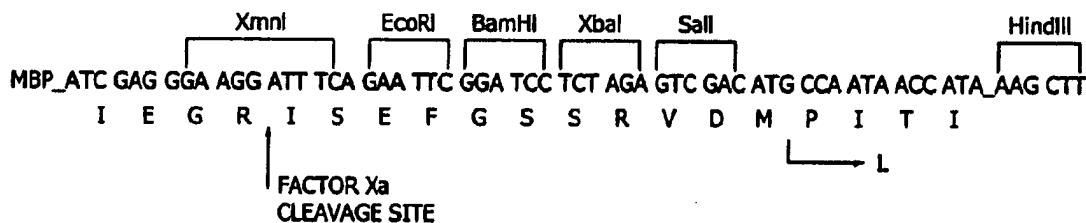
(21) Appl. No.: **09/751,053**(22) Filed: **Dec. 29, 2000**(51) Int. Cl.⁷ A01N 61/00; A01N 37/18; B61K 31/00; B61K 38/00; B61K 38/28

(52) U.S. Cl. 514/1; 514/2; 514/14

(58) Field of Search 514/1, 2, 14

(56) **References Cited****U.S. PATENT DOCUMENTS**5,223,408 A 6/1993 Goeddel et al.
5,595,880 A * 1/1997 Weinshank et al. 435/7.21
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6,641,820 B1 11/2003 Donovan**FOREIGN PATENT DOCUMENTS**

WO WO95/32738 12/1995

46 Claims, 1 Drawing Sheet

DOCUMENT-IDENTIFIER: US 5939070 A

TITLE: Hybrid botulinal neurotoxins

CLAIMS:

1. A hybrid botulinal neurotoxin comprising:

(a) a botulinal neurotoxin light chain; and

(b) a botulinal neurotoxin heavy chain,

wherein the light chain and heavy chain are not of the same serotype and wherein the light and heavy chains are linked by a heterobifunctional thiol/amine linker and wherein the specific toxicity of the neurotoxin is at least 10.sup.6 LD.sub.50 /mg protein in vivo.

2. The neurotoxin of claim 1 wherein the heavy chain or light chain is isolated from a native botulinal neurotoxin molecule.

3. The neurotoxin of claim 1 wherein the heavy chain or light chain is obtained from a recombinant gene construct.

4. The neurotoxin of claim 1 wherein the heavy and light chains are obtained from recombinant gene constructs.

5. A hybrid botulinal neurotoxin comprising light and heavy chains, which comprise botulinal neurotoxin catalytic, channel forming and receptor binding functional domains, wherein at least two functional domains are from botulinal neurotoxins of different serotypes and wherein the light and heavy chains are linked by a heterobifunctional thiol/amine linker and wherein the specific toxicity of the neurotoxin is at least 10.sup.6 LD.sub.50 /mg protein in vivo.

6. The neurotoxin of claim 5 wherein at least one of the functional domains is isolated from a native botulinal neurotoxin molecule.

7. The neurotoxin of claim 5 wherein at least one of the functional domains is isolated from a recombinant gene construct.

8. The neurotoxin of claim 5 wherein the heavy and light chains are obtained from recombinant gene constructs.

9. A pharmaceutical composition comprising the neurotoxin of claim 1.

10. A pharmaceutical composition comprising the neurotoxin of claim 5.

11. A method for creating a hybrid neurotoxin comprising the steps of:

(a) isolating botulinal neurotoxin heavy and light chains from native neurotoxin molecules or a recombinant gene construct; and

linking the heavy and light chains into a hybrid neurotoxin with a heterobifunctional thiol/amine linker

wherein the heavy and light chains are not of the same serotype and wherein the specific toxicity of the neurotoxin is at least 10.sup.6 LD.sub.50 /mg protein in vivo.

12. The method of claim 11 wherein the heavy and light chains are



US005939070A

United States Patent [19]
Johnson et al.

[11] **Patent Number:** **5,939,070**
 [45] **Date of Patent:** **Aug. 17, 1999**

[54] **HYBRID BOTULINAL NEUROTOXINS**

[75] **Inventors:** **Eric A. Johnson**, Madison; **Michael C. Goodnough**, Stoughton; **Marite Bradshaw**, Madison, all of Wis.

[73] **Assignee:** **Wisconsin Alumni Research Foundation**, Madison, Wis.

[21] **Appl. No.:** **08/739,477**

[22] **Filed:** **Oct. 28, 1996**

[51] **Int. Cl.⁶** **A61K 39/385**; **A61K 39/08**;
 C12P 21/06; C12N 9/52

[52] **U.S. Cl.** **424/194.1**; **424/239.1**;
 435/220; 435/842; 530/350; 530/402; 530/412;
 530/825; 536/23.2; 536/23.7; 514/12

[58] **Field of Search** 435/69.1, 172.3,
 435/220, 252.3, 320.1, 842; 514/12; 530/350,
 402, 412, 825; 536/23.2, 23.4, 23.7; 424/247.1,
 194.1, 239.1

[56] **References Cited**

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Primary Examiner—Rebecca E. Prouty

Assistant Examiner—Elizabeth Slobodyansky

Attorney, Agent, or Firm—Quarles & Brady LLP

[57] **ABSTRACT**

A hybrid botulinal neurotoxin is disclosed. In one embodiment, the neurotoxin comprises a combination of a botulinal neurotoxin heavy chain and light chain, wherein the light chain and heavy chain are not of the same serotype. A method for creating hybrid neurotoxins comprised of different functional domains is also disclosed.

14 Claims, 1 Drawing Sheet

: Entry 38 of 83

File: USPT

Mar 20, 2001

DOCUMENT-IDENTIFIER: US 6203794 B1

TITLE: Modification of clostridial toxins for use as transport proteins

CLAIMS:

1. A composition comprising,

a) an inactive Clostridial neurotoxin comprisingi) a light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species, wherein said light chain is inactivated by at least one said amino acid mutation, andii) an unaltered Clostridial neurotoxin heavy chain which has binding specificity for a target nerve cell; andb) a drug or other bioactive molecule joined to the inactivated light chain of said inactive neurotoxin,wherein said inactive neurotoxin is internalizable by said target nerve cell.2. The composition of claim 1, wherein said inactive neurotoxin comprises an inactive form of a toxin selected from the group consisting of: tetanus toxin, botulinum toxin A, botulinum toxin B, botulinum toxin C, botulinum toxin D, botulinum toxin E, botulinum toxin F, and botulinum toxin G.3. The composition of claim 2 wherein said inactive Clostridial neurotoxin is selected from the group consisting of a tetanus toxin comprising a modification of Glu.sup.224, a botulinum A toxin comprising a modification at His.sup.227, a botulinum A toxin comprising a modification at Glu.sup.224, a botulinum toxin other than botulinum toxin A comprising a modification at a site corresponding to His.sup.227 of botulinum toxin A, and a botulinum toxin other than botulinum toxin A comprising a modification at a site corresponding to Glu.sup.224 of botulinum toxin A.

4. A pharmaceutical composition for treatment of a neuromuscular dysfunction in a mammal, comprising:

a) an inactive Clostridial neurotoxin comprisingi) a light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species, wherein said light chain is inactivated by at least one said amino acid mutation, andii) an unaltered Clostridial neurotoxin heavy chain which has binding specificity for a target nerve cell; andb) a drug or other bioactive molecule joined to the inactivated light chain of said inactive neurotoxin,wherein said inactive neurotoxin is internalizable by said target nerve cell, and a pharmaceutically acceptable excipient.

8. The composition of either of claims 1 or 5 wherein said drug or other bioactive molecule is an active ingredient for treatment of botulism or tetanus.

9. The composition of either of claims 1 or 5 wherein said drug or other bioactive molecule is selected from the group consisting of:

- a) a GABA agonist,
- b) a neuronal calcium channel agonist,
- c) an adenosine agonist,
- d) a glutamate antagonist,
- e) a protein synthesis toxin,
- f) a zinc-dependent protease inhibitor,
- g) a neuronal growth factor,
- h) an antiviral agent,
- i) a nicotinic antagonist,



US006203794B1

(12) United States Patent
Dolly et al.**(10) Patent No.: US 6,203,794 B1**
(45) Date of Patent: *Mar. 20, 2001**(54) MODIFICATION OF CLOSTRIDIAL TOXINS
FOR USE AS TRANSPORT PROTEINS****(75) Inventors:** James Oliver Dolly, Cheam (GB); Kei
Roger Aoki, Laguna Hills, CA (US);
Larry Allen Wheeler, Irvine, CA (US);
Michael Elwood Garst, Newport
Beach, CA (US)**(73) Assignee:** Allergan Sales, Inc.**(*) Notice:** This patent issued on a continued pro-
secution application filed under 37 CFR
1.53(d), and is subject to the twenty year
patent term provisions of 35 U.S.C.
154(a)(2).Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.**(21) Appl. No.:** 08/750,101**(22) PCT Filed:** May 31, 1995**(86) PCT No.:** PCT/GB95/01253

§ 371 Date: May 1, 1997

§ 102(c) Date: May 1, 1997

(87) PCT Pub. No.: WO95/32738

PCT Pub. Date: Dec. 7, 1995

(30) Foreign Application Priority DataMay 31, 1994 (GB) 9410870
May 31, 1994 (GB) 9410871**(51) Int. Cl.⁷** A61K 39/395; A61K 39/02;
A61K 38/00; C07K 14/00**(52) U.S. Cl.** 424/184.1; 424/234.1;
424/235.1; 424/236.1; 424/239.1; 424/247.1;
424/183.1; 424/178.1; 424/179.1; 424/164.1;
424/167.1; 424/832; 530/300; 530/350**(58) Field of Search** 424/184.1, 234.1,
424/235.1, 236.1, 239.1, 247.1, 183.1, 178.1,
179.1, 164.1, 167.1, 832; 530/300, 350**(56) References Cited****U.S. PATENT DOCUMENTS**4,594,336 * 6/1986 Bizzini .
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5,204,097 * 4/1993 Arnon et al. .
5,437,291 * 8/1995 Pasricha et al. .
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5,562,907 * 10/1996 Arnon .
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5,762,934 * 6/1998 Williams et al. .
5,814,477 * 9/1998 Williams et al. .
5,919,665 * 7/1999 Williams .
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(List continued on next page.)

Primary Examiner—Nita Minnifield*(74) Attorney, Agent, or Firm*—Carlos A. Fisher; Robert J.
Baran; Martin A. Voet**(57) ABSTRACT**A chemical conjugate for treating a nerve cell related dis-
order is provided. The conjugate includes an active or
inactive Clostridial toxin having specificity for a target nerve
cell. The toxin is conjugated to a drug or other bioactive
molecule without affecting the toxin's ability to enter the
target nerve cell.**14 Claims, 9 Drawing Sheets**

✓
US-PAT-NO: 6395513

DOCUMENT-IDENTIFIER: US 6395513 B1

TITLE: Clostridial toxin derivatives able to modify peripheral sensory afferent functions

DATE-ISSUED: May 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Foster; Keith Alan	Wiltshire			GB
Duggan; Michael John	London			GB
Shone; Clifford Charles	Wiltshire			GB

US-CL-CURRENT: 435/69.3; 435/69.1, 435/69.7, 530/350

CLAIMS:

What is claimed is:

1. A method for preparing an agent in the form of a fusion protein, which agent agent binds to a peripheral sensory afferent,

the agent comprising a Targeting Moiety (TM) coupled to a modified clostridial neurotoxin in which the TM comprises a ligand to a cell-surface binding site present on a primary sensory afferent and is capable of functionally interacting with the binding site causing a physical association between the agent and the surface of the primary sensory afferent,

wherein the heavy chain (H-chain) of the clostridial neurotoxin is removed or modified to remove or reduce its native binding affinity for motor neurons,

and the light chain (L-chain) of the clostridial neurotoxin or a fragment thereof retains a protease activity specific for components of the neurosecretory machinery,

the TM and the modified H-chain, if present, forming a molecule which introduces the L-chain or fragment thereof into the cytosol of a primary sensory afferent, thereby inhibiting the transmission of signals between a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent;

said method comprising expressing in a host organism a genetic construct which codes for the agent.

2. A method according to claim 1 which further comprises constructing the genetic construct and transforming the host with said construct.

3. A method according to claim 1, wherein the genetic construct includes a sequence encoding a spacer molecule by which the modified clostridial neurotoxin, or a fragment thereof, is coupled to the TM.

4. A method according to claim 3, wherein the spacer molecule is selected from

the group consisting of (SEQ ID NO: 11) PPPIEGR, collagen-like spacer, and trypsin-sensitive diphtheria toxin peptide.

5. A method according to claim 1, wherein the nucleic acid sequence of the genetic construct is modified in accordance with the codon bias of the host cell.

6. A method according to claim 1, wherein the genetic construct incorporates a nucleic acid sequence encoding an affinity tag to facilitate purification of the assembled toxin.

7. An agent which binds to a peripheral sensory afferent and has been obtained in the form of a fusion protein by the method according to claim 1, said agent comprising

a Targeting Moiety (TM) coupled to a modified clostridial neurotoxin in which the TM comprises a ligand to a cell-surface binding site present on a primary sensory afferent and is capable of functionally interacting with the binding site causing a physical association between the agent and the surface of the primary sensory afferent,

wherein the heavy chain (H-chain) of the clostridial neurotoxin is removed or modified to remove or reduce its native binding affinity for motor neurons,

and the light chain (L-chain) of the clostridial neurotoxin or a fragment thereof retains a protease activity specific for components of the neurosecretory machinery,

the TM and the modified H-chain, if present, forming a molecule which introduces the L-chain or fragment thereof into the cytosol of a primary sensory afferent, thereby inhibiting the transmission of signals between a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent.

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US006395513B1

(12) **United States Patent**
Foster et al.

(10) **Patent No.:** US 6,395,513 B1
(45) **Date of Patent:** *May 28, 2002

(54) **CLOSTRIDIAL TOXIN DERIVATIVES ABLE TO MODIFY PERIPHERAL SENSORY AFFERENT FUNCTIONS**

(75) **Inventors:** Keith Alan Foster, Wiltshire; Michael John Duggan, London; Clifford Charles Shone, Wiltshire, all of (GB)

(73) **Assignees:** The Speywood Laboratory, Ltd., London; Microbiological Research Authority, Wiltshire, both of (GB)

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) **Appl. No.:** 09/447,356

(22) **Filed:** Nov. 22, 1999

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/945,037, filed as application No. PCT/GB96/00916 on Apr. 16, 1996, now Pat. No. 5,989,545.

(30) **Foreign Application Priority Data**

Apr. 21, 1995 (GB) 9508204

(51) **Int. Cl.⁷** C12N 15/62; C12N 15/09; C12P 21/00; C07K 19/00

(52) **U.S. Cl.** 435/69.3; 435/69.1; 435/69.7; 530/350

(58) **Field of Search** 435/69.1, 69.3, 435/69.7; 530/350

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Primary Examiner—Mary E. Mosher

(74) *Attorney, Agent, or Firm*—Foley & Lardner

(57) **ABSTRACT**

The invention relates to an agent specific for peripheral sensory afferents. The agent may inhibit the transmission of signals between a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent. The agent may be used in or as a pharmaceutical for the treatment of pain, particularly chronic pain.

7 Claims, 4 Drawing Sheets

: Entry 27 of 47

File: USPT

Mar 20, 2001

DOCUMENT-IDENTIFIER: US 6203794 B1

TITLE: Modification of clostridial toxins for use as transport proteins

CLAIMS:

1. A composition comprising,

a) an inactive Clostridial neurotoxin comprising

i) a light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species, wherein said light chain is inactivated by at least one said amino acid mutation, and

ii) an unaltered Clostridial neurotoxin heavy chain which has binding specificity for a target nerve cell; and

b) a drug or other bioactive molecule joined to the inactivated light chain of said inactive neurotoxin,

wherein said inactive neurotoxin is internalizable by said target nerve cell.

2. The composition of claim 1, wherein said inactive neurotoxin comprises an inactive form of a toxin selected from the group consisting of: tetanus toxin, botulinum toxin A, botulinum toxin B, botulinum toxin C, botulinum toxin D, botulinum toxin E, botulinum toxin F, and botulinum toxin G.

3. The composition of claim 2 wherein said inactive Clostridial neurotoxin is selected from the group consisting of a tetanus toxin comprising a modification of Glu.sup.224, a botulinum A toxin comprising a modification at His.sup.227, a botulinum A toxin comprising a modification at Glu.sup.224, a botulinum toxin other than botulinum toxin A comprising a modification at a site corresponding to His.sup.227 of botulinum toxin A, and a botulinum toxin other than botulinum toxin A comprising a modification at a site corresponding to Glu.sup.224 of botulinum toxin A.

4. A pharmaceutical composition for treatment of a neuromuscular dysfunction in a mammal, comprising:

a) an inactive Clostridial neurotoxin comprising

i) a light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species, wherein said light chain is inactivated by at least one said amino acid mutation, and

ii) an unaltered Clostridial neurotoxin heavy chain which has binding specificity for a target nerve cell; and

b) a drug or other bioactive molecule joined to the inactivated light chain of said inactive neurotoxin,

wherein said inactive neurotoxin is internalizable by said target nerve cell, and a pharmaceutically acceptable excipient.

10. A method for treating a mammal having acute botulinum poisoning, comprising:

introducing into said mammal an effective quantity of a pharmaceutically active solution comprising

a) an inactive Clostridial neurotoxin comprising

i) a light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species, wherein said light chain is inactivated by at least one said amino acid mutation, and

ii) an unaltered Clostridial neurotoxin heavy chain which has binding specificity for a target nerve cell; and

b) a drug or other bioactive molecule joined to the inactivated light chain of said inactive neurotoxin,

wherein said inactive neurotoxin is internalizable by said target nerve cell, thereby lessening the effects of said acute botulinum poisoning.

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US006203794B1

(12) **United States Patent**
Dolly et al.(10) Patent No.: **US 6,203,794 B1**
(45) Date of Patent: ***Mar. 20, 2001**(54) **MODIFICATION OF CLOSTRIDIAL TOXINS
FOR USE AS TRANSPORT PROTEINS**(75) Inventors: **James Oliver Dolly, Cheam (GB); Kei
Roger Aoki, Laguna Hills, CA (US);
Larry Allen Wheeler, Irvine, CA (US);
Michael Elwood Garst, Newport
Beach, CA (US)**(73) Assignee: **Allergan Sales, Inc.**

(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/750,101**(22) PCT Filed: **May 31, 1995**(86) PCT No.: **PCT/GB95/01253**§ 371 Date: **May 1, 1997**§ 102(e) Date: **May 1, 1997**(87) PCT Pub. No.: **WO95/32738**PCT Pub. Date: **Dec. 7, 1995**(30) **Foreign Application Priority Data**May 31, 1994 (GB) 9410870
May 31, 1994 (GB) 9410871(51) Int. Cl.⁷ **A61K 39/395; A61K 39/02;
A61K 38/00; C07K 14/00**(52) U.S. Cl. **424/184.1; 424/234.1;
424/235.1; 424/236.1; 424/239.1; 424/247.1;
424/183.1; 424/178.1; 424/179.1; 424/164.1;
424/167.1; 424/832; 530/300; 530/350**(58) Field of Search **424/184.1, 234.1,
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179.1, 164.1, 167.1, 832; 530/300, 350**(56) **References Cited****U.S. PATENT DOCUMENTS**4,594,336 * 6/1986 Bizzini .
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5,965,699 * 10/1999 Schmidt et al. .**FOREIGN PATENT DOCUMENTS**0254905 3/1988 (EP) .
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9421300 9/1994 (WO) .**OTHER PUBLICATIONS**Wadsworth et al, Biochem. J., 268:123-128, 1990.*
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Höhne-Zell et al, Endocrinology, 138/12:5518-5526, 1997.*
Pellizzari et al, FEBS Letters, 409:339-342, 1997.*

(List continued on next page.)

Primary Examiner—Nita Minnifield(74) *Attorney, Agent, or Firm*—Carlos A. Fisher; Robert J. Baran; Martin A. Voet(57) **ABSTRACT**

A chemical conjugate for treating a nerve cell related disorder is provided. The conjugate includes an active or inactive Clostridial toxin having specificity for a target nerve cell. The toxin is conjugated to a drug or other bioactive molecule without affecting the toxin's ability to enter the target nerve cell.

14 Claims, 9 Drawing Sheets



US006395513B1

(12) **United States Patent**
Foster et al.

(10) **Patent No.:** US 6,395,513 B1
(45) **Date of Patent:** *May 28, 2002

(54) **CLOSTRIDIAL TOXIN DERIVATIVES ABLE TO MODIFY PERIPHERAL SENSORY AFFERENT FUNCTIONS**

(75) **Inventors:** Keith Alan Foster, Wiltshire; Michael John Duggan, London; Clifford Charles Shone, Wiltshire, all of (GB)

(73) **Assignees:** The Speywood Laboratory, Ltd., London; Microbiological Research Authority, Wiltshire, both of (GB)

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) **Appl. No.:** 09/447,356

(22) **Filed:** Nov. 22, 1999

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/945,037, filed as application No. PCT/GB96/00916 on Apr. 16, 1996, now Pat. No. 5,989,545.

(30) Foreign Application Priority Data

Apr. 21, 1995 (GB) 9508204

(51) **Int. Cl.⁷** C12N 15/62; C12N 15/09; C12P 21/00; C07K 19/00

(52) **U.S. Cl.** 435/69.3; 435/69.1; 435/69.7; 530/350

(58) **Field of Search** 435/69.1, 69.3, 435/69.7; 530/350

(56) References Cited

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5,989,545 A * 11/1999 Foster et al. 424/183.1

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WO 93/04191 3/1993

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John R. Murphy, Diphtheria-related peptide hormone gene fusions: A molecular genetic approach to chimeric toxin development, *Cancer Treatment Res*; 37:123-40.

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Primary Examiner—Mary E. Mosher

(74) *Attorney, Agent, or Firm*—Foley & Lardner

(57) ABSTRACT

The invention relates to an agent specific for peripheral sensory afferents. The agent may inhibit the transmission of signals between a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent. The agent may be used in or as a pharmaceutical for the treatment of pain, particularly chronic pain.

7 Claims, 4 Drawing Sheets

-continued

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What is claimed is:

1. A method for preparing an agent in the form of a fusion protein, which agent binds to a peripheral sensory afferent, the agent comprising a Targeting Moiety (TM) coupled to a modified clostridial neurotoxin in which the TM comprises a ligand to a cell-surface binding site present on a primary sensory afferent and is capable of functionally interacting with the binding site causing a physical association between the agent and the surface of the primary sensory afferent,

wherein the heavy chain (H-chain) of the clostridial neurotoxin is removed or modified to remove or reduce its native binding affinity for motor neurons, and the light chain (L-chain) of the clostridial neurotoxin or a fragment thereof retains a protease activity specific for components of the neurosecretory machinery, the TM and the modified H-chain, if present, forming a molecule which introduces the L-chain or fragment thereof into the cytosol of a primary sensory afferent, thereby inhibiting the transmission of signals between

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a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent; said method comprising expressing in a host organism a genetic construct which codes for the agent.

2. A method according to claim 1 which further comprises constructing the genetic construct and transforming the host with said construct.

3. A method according to claim 1, wherein the genetic construct includes a sequence encoding a spacer molecule by which the modified clostridial neurotoxin, or a fragment thereof, is coupled to the TM.

4. A method according to claim 3, wherein the spacer molecule is selected from the group consisting of (SEQ ID NO: 11) PPPIEGR, collagen-like spacer, and trypsin-sensitive diphtheria toxin peptide.

5. A method according to claim 1, wherein the nucleic acid sequence of the genetic construct is modified in accordance with the codon bias of the host cell.

6. A method according to claim 1, wherein the genetic construct incorporates a nucleic acid sequence encoding an affinity tag to facilitate purification of the assembled toxin.

7. An agent which binds to a peripheral sensory afferent and has been obtained in the form of a fusion protein by the method according to claim 1, said agent comprising

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a Targeting Moiety (TM) coupled to a modified clostridial neurotoxin in which the TM comprises a ligand to a cell-surface binding site present on a primary sensory afferent and is capable of functionally interacting with the binding site causing a physical association between the agent and the surface of the primary sensory afferent,

wherein the heavy chain (H-chain) of the clostridial neurotoxin is removed or modified to remove or reduce its native binding affinity for motor neurons,

and the light chain (L-chain) of the clostridial neurotoxin or a fragment thereof retains a protease activity specific for components of the neurosecretory machinery,

the TM and the modified H-chain, if present, forming a molecule which introduces the L-chain or fragment thereof into the cytosol of a primary sensory afferent, thereby inhibiting the transmission of signals between a primary sensory afferent and a projection neuron by controlling the release of at least one neurotransmitter or neuromodulator from the primary sensory afferent.

* * * * *

-IDENTIFIER: US 5965406 A

**** See image for Certificate of Correction ****

TITLE: Recombinant DNAs encoding three-part hybrid proteins

CLAIMS:

1. A recombinant DNA molecule encoding a hybrid protein comprising a first part, a second part, and a third part,
 - (a) wherein said first part comprises a portion of the binding domain of a cell-binding polypeptide ligand effective to cause said hybrid protein to bind to a cell of an animal;
 - (b) wherein said second part comprises a portion of a translocation domain of a naturally occurring protein selected from the group consisting of diphtheria toxin, botulinum neurotoxin, ricin, cholera toxin, LT toxin, C3 toxin, Shiga toxin, Shiga-like toxin, pertussis toxin and tetanus toxin, which translocates said third part across the cytoplasmic membrane into the cytosol of the cell; and
 - (c) wherein said third part comprises a polypeptide entity to be introduced into the cell, wherein said third part is non-native with respect to said naturally occurring protein of (b).
2. The recombinant DNA molecule of claim 1, wherein said first part comprises the binding domain of said cell-binding polypeptide ligand.
5. The recombinant DNA molecule of claim 1, wherein said cell-binding polypeptide ligand is an antigen-binding, single-chain analog of a monoclonal antibody.
7. The recombinant DNA molecule of claim 1, wherein said first part comprises a portion of the binding domain of a polypeptide toxin.
8. The recombinant DNA molecule of claim 1, wherein said polypeptide entity of (c) is an antigen-binding, single-chain analog of a monoclonal antibody.
9. The recombinant DNA molecule of claim 1, wherein said polypeptide entity of (c) comprises an enzymatically active portion of an enzyme.
10. The recombinant DNA molecule of claim 1, wherein said polypeptide entity of (c) comprises an enzymatically active portion of a protease.
11. The recombinant DNA molecule of claim 1, wherein said polypeptide entity of (c) comprises an enzymatically active portion of a nuclease.
12. The recombinant DNA molecule of claim 1, wherein said polypeptide entity of (c) comprises an enzymatically active portion of a toxin.
14. The recombinant DNA molecule of claim 1, wherein said second part comprises a portion of the translocation domain of Shiga-like toxin.
15. The recombinant DNA molecule of claim 1, wherein said third part comprises an enzymatically active portion of Shiga-like toxin A, and wherein said second and third parts are connected via a proteolytically-sensitive disulfide-loop.

22. The recombinant DNA molecule of claim 15, wherein said first part comprises the binding domain of interleukin II.

29. A recombinant DNA molecule encoding a hybrid protein comprising a first part, a second part and a third part,

(a) wherein said first part comprises a portion of the binding domain of a cell-binding polypeptide ligand effective to cause said hybrid protein to bind to a cell of an animal;

(b) wherein said second part comprises a portion of the translocation domain of diphtheria toxin which translocates said third part across the cytoplasmic membrane and into the cytosol of the cell; and

(c) wherein said third part comprises a polypeptide entity to be introduced into the cell, wherein said polypeptide entity is non-native with respect to said diphtheria toxin.

30. The recombinant DNA molecule of claim 29, wherein said first part comprises a portion of the binding domain of interleukin II effective to cause said hybrid protein to bind to an interleukin II receptor-bearing cell.

31. The recombinant DNA molecule of claim 29, wherein said first part comprises a portion of the binding domain of diphtheria toxin.

32. The recombinant DNA molecule of claim 29, wherein said first part comprises a portion of the binding domain of EGF.

33. The recombinant DNA molecule of claim 29, wherein said second part comprises Fragment B' of diphtheria toxin illustrated in FIG. 3.

34. The recombinant DNA molecule of claim 29, wherein said third part comprises an enzymatically active portion of cholera toxin.

35. The recombinant DNA molecule of claim 29, wherein said third part comprises an enzymatically active portion of ricin toxin.

36. The recombinant DNA molecule of claim 29, wherein said third part comprises an enzymatically active portion of Shiga-like toxin.

45. A method of preparing a hybrid protein comprising a first part, a second part, and a third part,

(a) wherein said first part comprises a portion of the binding domain of a cell-binding polypeptide ligand effective to cause said hybrid protein to bind to a cell of a animal;

(b) wherein said second part comprises a portion of a translocation domain of a naturally occurring protein selected from the group consisting of diphtheria toxin, botulinum neurotoxin, ricin, cholera toxin, LT toxin, C3 toxin, Shiga toxin, Shiga-like toxin, pertussis toxin and tetanus toxin, which translocates said third part across the cytoplasmic membrane into the cytosol of the cell; and

(c) wherein said third part comprises a polypeptide entity to be introduced into the cell, wherein said third part is non-native with respect to said naturally occurring protein of (b) comprising the steps of:

providing a cell transformed with a recombinant DNA molecule encoding the hybrid protein, and
culturing the transformed cell to allow expression of the recombinant DNA molecule such that the hybrid protein is produced.

48. A method of preparing a hybrid protein comprising a first part, a second part, and a third part,

(a) wherein said first part comprises a portion of the binding domain of a cell-binding polypeptide ligand effective to cause the hybrid protein to bind to a cell of an animal;

(b) wherein said second part comprises a portion of a translocation domain of diphtheria toxin which translocates said third part across the cytoplasmic membrane into the cytosol of the cell; and

(c) wherein said third part comprises a polypeptide entity to be introduced into the cell, wherein said third part is non-native with respect to said diphtheria toxin, comprising the steps of:

providing a cell transformed with a recombinant DNA molecule encoding the hybrid protein, and

culturing the transformed cell to allow expression of the recombinant DNA molecule such that the hybrid protein is produced.



US005965406A

United States Patent [19]
Murphy

[11] **Patent Number:** **5,965,406**
 [45] **Date of Patent:** **Oct. 12, 1999**

[54] **RECOMBINANT DNAS ENCODING THREE-PART HYBRID PROTEINS**

[75] **Inventor:** John R. Murphy, Wayland, Mass.

[73] **Assignee:** Seragen, Inc., Hopkinton, Mass.

[21] **Appl. No.:** 08/488,246

[22] **Filed:** Jun. 7, 1995

Related U.S. Application Data

[62] Division of application No. 08/102,387, Aug. 4, 1993, Pat. No. 5,668,255, which is a continuation of application No. 07/722,484, Jun. 27, 1991, abandoned, which is a continuation-in-part of application No. 07/538,276, Jun. 14, 1990, abandoned, which is a continuation-in-part of application No. 07/456,095, Dec. 22, 1989, abandoned, which is a continuation-in-part of application No. 06/742,554, Jun. 7, 1985, abandoned, which is a continuation-in-part of application No. 06/726,808, Apr. 25, 1985, abandoned, which is a continuation of application No. 06/618,199, Jun. 7, 1984, abandoned.

[51] **Int. Cl.⁶** C12N 1/21; C12N 15/12; C12N 15/63; C12P 21/02

[52] **U.S. Cl.** 435/69.7; 435/252.33; 435/320.1; 536/23.4

[58] **Field of Search** 530/350; 435/69.7; 435/252.33, 320.1; 536/23.4, 23.51

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Primary Examiner—Nancy Degen

Assistant Examiner—Robert Schwartzman

Attorney, Agent, or Firm—Lerner, David, Littenberg, Krumholz & Mentlik, LLP

[57] **ABSTRACT**

Disclosed is a recombinant DNA molecule encoding a hybrid protein comprising a first part, a second part, and a third part,

- (a) wherein said first part comprises a portion of the binding domain of a cell-binding polypeptide ligand effective to cause said hybrid protein to bind to a cell of an animal;
- (b) wherein said second part comprises a portion of a translocation domain of naturally occurring protein selected from the group consisting of diphtheria toxin, botulinum neurotoxin, ricin, cholera toxin, LT toxin, C3 toxin, Shiga toxin, Shiga-like toxin, pertussis toxin and tetanus toxin, which translocates said third part across the cytoplasmic membrane into the cytosol of the cell; and
- (c) wherein said third part comprises a polypeptide entity to be introduced into the cell, wherein said third part is non-native with respect to said naturally occurring protein of (b).

51 Claims, 19 Drawing Sheets

Entry 31 of 47

File: USPT

Apr 18, 2000

DOCUMENT-IDENTIFIER: US 6051239 A

TITLE: Compositions and methods for systemic delivery of oral vaccines and therapeutic agents

CLAIMS:

1. A modified botulinum toxin comprising a botulinum toxin capable of translocating from the gut to the general circulation and a selected antigen, wherein said botulinum toxin is altered to be nontoxic by mutating or deleting amino acids in the light chain of the botulinum toxin.
2. A method of protecting an animal against botulism comprising administering orally to an animal a modified botulinum toxin and a pharmaceutically acceptable vehicle, wherein said botulinum toxin is altered to be nontoxic by mutating or deleting amino acids in the light chain of the botulinum toxin.



US006051239A

United States Patent [19][11] **Patent Number:** **6,051,239****Simpson et al.**[45] **Date of Patent:** **Apr. 18, 2000**[54] **COMPOSITIONS AND METHODS FOR SYSTEMIC DELIVERY OF ORAL VACCINES AND THERAPEUTIC AGENTS**[75] **Inventors:** Lance Simpson, Moorestown; Nikita Kiyatkin, Cherry Hill, both of N.J.; Andrew Maksymowych, Gulph Mills, Pa.[73] **Assignee:** Thomas Jefferson University, Philadelphia, Pa.[21] **Appl. No.:** 08/954,302[22] **Filed:** Oct. 20, 1997[51] **Int. Cl.⁷** A61K 39/08; C07K 14/33; C07K 19/00[52] **U.S. Cl.** 424/239.1; 424/832; 424/190.1; 424/192.1; 530/350; 435/69.3; 435/69.7[58] **Field of Search** 424/184.1, 192.1, 424/247.11, 183.1, 94.1, 239.1, 832, 190.1, 94.63; 530/350; 435/183, 212, 69.3, 69.7[56] **References Cited****U.S. PATENT DOCUMENTS**

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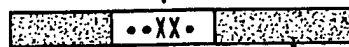
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ABSTRACT

Compositions and methods of oral delivery of an antigen or therapeutic agent to the general circulation using a modified botulinum toxin which is capable of translocating from the gut to the general circulation but which is altered to be nontoxic are provided.

2 Claims, 1 Drawing Sheet

LIGHT CHAIN (~50,000 Da)
with **modified ZINC BINDING DOMAIN**



S~S

HEAVY CHAIN (~100,000 Da)

Entry 27 of 47

File: USPT

Mar 20, 2001

DOCUMENT-IDENTIFIER: US 6203794 B1

TITLE: Modification of clostridial toxins for use as transport proteins

CLAIMS:

1. A composition comprising,

a) an inactive Clostridial neurotoxin comprising

i) a light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species, wherein said light chain is inactivated by at least one said amino acid mutation, andii) an unaltered Clostridial neurotoxin heavy chain which has binding specificity for a target nerve cell; andb) a drug or other bioactive molecule joined to the inactivated light chain of said inactive neurotoxin,

wherein said inactive neurotoxin is internalizable by said target nerve cell.

2. The composition of claim 1, wherein said inactive neurotoxin comprises an inactive form of a toxin selected from the group consisting of: tetanus toxin, botulinum toxin A, botulinum toxin B, botulinum toxin C, botulinum toxin D, botulinum toxin E, botulinum toxin F, and botulinum toxin G.3. The composition of claim 2 wherein said inactive Clostridial neurotoxin is selected from the group consisting of a tetanus toxin comprising a modification of Glu.sup.224, a botulinum A toxin comprising a modification at His.sup.227, a botulinum A toxin comprising a modification at Glu.sup.224, a botulinum toxin other than botulinum toxin A comprising a modification at a site corresponding to His.sup.227 of botulinum toxin A, and a botulinum toxin other than botulinum toxin A comprising a modification at a site corresponding to Glu.sup.224 of botulinum toxin A.

4. A pharmaceutical composition for treatment of a neuromuscular dysfunction in a mammal, comprising:

a) an inactive Clostridial neurotoxin comprising

i) a light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species, wherein said light chain is inactivated by at least one said amino acid mutation, andii) an unaltered Clostridial neurotoxin heavy chain which has binding specificity for a target nerve cell; andb) a drug or other bioactive molecule joined to the inactivated light chain of said inactive neurotoxin,

wherein said inactive neurotoxin is internalizable by said target nerve cell, and a pharmaceutically acceptable excipient.

10. A method for treating a mammal having acute botulinum poisoning, comprising:

introducing into said mammal an effective quantity of a pharmaceutically active solution comprising

a) an inactive Clostridial neurotoxin comprising

i) a light chain containing one or more amino acid sequence mutation as compared to the amino acid sequence of the light chain of a wild-type Clostridial neurotoxin of the same type and from the same species, wherein said light chain is inactivated by at least one said amino acid mutation, and

ii) an unaltered Clostridial neurotoxin heavy chain which has binding specificity for a target nerve cell; and

b) a drug or other bioactive molecule joined to the inactivated light chain of said inactive neurotoxin,

wherein said inactive neurotoxin is internalizable by said target nerve cell, thereby lessening the effects of said acute botulinum poisoning.

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US006203794B1

(12) **United States Patent**
Dolly et al.(10) **Patent No.:** **US 6,203,794 B1**
(45) **Date of Patent:** ***Mar. 20, 2001**(54) **MODIFICATION OF CLOSTRIDIAL TOXINS
FOR USE AS TRANSPORT PROTEINS**(75) **Inventors:** James Oliver Dolly, Cheam (GB); Kel
Roger Aoki, Laguna Hills, CA (US);
Larry Allen Wheeler, Irvine, CA (US);
Michael Elwood Garst, Newport
Beach, CA (US)(73) **Assignee:** Allergan Sales, Inc.(*) **Notice:** This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) **Appl. No.:** **08/750,101**(22) **PCT Filed:** **May 31, 1995**(86) **PCT No.:** **PCT/GB95/01253**§ 371 Date: **May 1, 1997**§ 102(c) Date: **May 1, 1997**(87) **PCT Pub. No.:** **WO95/32738****PCT Pub. Date: Dec. 7, 1995**(30) **Foreign Application Priority Data**May 31, 1994 (GB) 9410870
May 31, 1994 (GB) 9410871(51) **Int. Cl.⁷** **A61K 39/395; A61K 39/02;**
A61K 38/00; C07K 14/00(52) **U.S. Cl.** **424/184.1; 424/234.1;**
424/235.1; 424/236.1; 424/239.1; 424/247.1;
424/183.1; 424/178.1; 424/179.1; 424/164.1;
424/167.1; 424/832; 530/300; 530/350(58) **Field of Search** **424/184.1, 234.1,**
424/235.1, 236.1, 239.1, 247.1, 183.1, 178.1,
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Primary Examiner—Nita Minnifield(74) *Attorney, Agent, or Firm*—Carlos A. Fisher; Robert J. Baran; Martin A. Voet(57) **ABSTRACT**

A chemical conjugate for treating a nerve cell related disorder is provided. The conjugate includes an active or inactive Clostridial toxin having specificity for a target nerve cell. The toxin is conjugated to a drug or other bioactive molecule without affecting the toxin's ability to enter the target nerve cell.

14 Claims, 9 Drawing Sheets

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L3: Entry 4 of 47

File: USPT

Nov 23, 2004

DOCUMENT-IDENTIFIER: US 6822076 B2

TITLE: Hybrid protein for inhibiting the degranulation of mastocytes and the use thereof

CLAIMS:

1. A hybrid protein comprising: (i) protein capable of binding to a receptor at least one cell type selected from the group consisting of mastocytes and basophils and of being endocyted by the at least one cell type selected from the group consisting of the mastocytes and basophils; (ii) a protease capable of cleaning one or more secreted proteins of the at least one cell type selected from the group consisting of the mastocytes and basophils so as to inhibit the secretion process without killing the at least one cell type selected from the group consisting of the mastocytes and basophils, wherein the protease (ii) is selected from the group consisting of: light chain of a Clostridium botulinum neurotoxin; proteolytically active fragment of the light chain of a Clostridium botulinum neurotoxin containing an amino acid sequence of SEQ ID NO:1 His-Xaa-Xaa-Xaa-His-Xaa-Xaa-His, wherein Xaa is any amino acid. light chain of the tetanus toxin (TeNT); proteolytically active fragment of the light chain of the tetanus toxin containing an amino acid sequence of SEQ ID NO:2 His-Asp-Leu-Ile-His-Val-Leu-His; IgA protease of Neisseria gonorrhoeae; and proteolytic domain of the IgA protease of Neisseria gonorrhoeae.
3. The hybrid protein according to claim 1, wherein the protein (i) is selected from the group consisting of: IgE; IgE fragment; IgE Fc fragment; antibody against IgE receptor of the at least one of the mastocytes and basophils; fragment of the antibody against the IgE receptor of the at least one of the mastocytes and basophils; antibody against mastocyte-specific potassium channel; and MCD (mast cell degranulating) peptide.
4. The hybrid protein according to claim 3, wherein the fragment of the antibody against the IgE receptor of the at least one of the mastocytes and basophils is a Fab fragment.
5. The hybrid protein according to claim 3, further comprising the N-terminal portion of a heavy chain of a neurotoxin (H.sub.N fragment) or a fragment thereof in addition to the light chain of a Clostridium botulinum neurotoxin or of the tetanus toxin.
6. A hybrid protein comprising: (i) a protein capable of binding to a receptor of at least one cell type selected from the group consisting of mastocytes and basophils and of being endocyted by the at least one cell type selected from the group consisting of the mastocytes and basophils, wherein the protein is selected from the group consisting of: IgE; IgE fragment; IgE Fc fragment; antibody against IgE receptor of the at least one cell type selected from the group consisting of the mastocytes and basophils; fragment of the antibody against the IgE receptor of the at least one cell type selected from the group consisting of the mastocytes and basophils; antibody against mastocyte-specific potassium channel; and MCD (mast cell degranulating) peptide; and (ii) a protease capable of cleaving one or more secreted of the at least one cell type selected from the group consisting of the

mastocytes and basophils so as to inhibit the secretion process without killing the at least one cell type selected from the group consisting of the mastocytes and basophils, wherein the protease is selected from the group consisting of: light chain of a Clostridium botulinum toxin neurotoxin; proteolytically active fragment of the light chain of a Clostridium botulinum neurotoxin containing an amino acid sequence of SEQ ID NO:1 His-Xaa-Xaa-Xaa-His-Xaa-Xaa-His wherein Xaa is any amino acid; light chain of the tetanus toxin (TeNT); proteolytically active fragment of the light chain of the tetanus toxin containing an amino acid sequence of SEQ ID NO :2 His-Asp-Leu-Ile-His-Val- Leu-His; IgA protease of Neisseria gonorrhoeae; and proteolytic domain of the IgA protease of Neisseria gonorrhoeae.

7. The hybrid protein according to claim 6, wherein the fragment of the antibody against the IgE receptor of the at least one of the mastocytes and basophils is a Fab fragment.

9. The hybrid protein according to claim 6, further comprising the N-terminal portion of a heavy chain of a botulinum neurotoxin or a tetanus toxin (H.sub.N fragment) or a fragment of the N-terminal portion of the heavy chain of the botulinum neurotoxin or the tetanus toxin in addition to the light chain of the Clostridium botulinum neurotoxin or of the tetanus toxin.

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(12) **United States Patent**
Bigalke et al.

(10) **Patent No.:** **US 6,822,076 B2**
(45) **Date of Patent:** **Nov. 23, 2004**

(54) **HYBRID PROTEIN FOR INHIBITING THE
DEGRANULATION OF MASTOCYTES AND
THE USE THEREOF**

(75) **Inventors:** **Hans Bigalke, Hannover, DE (US);
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(*) **Notice:** Subject to any disclaimer, the term of this
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(30) **Foreign Application Priority Data**

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(52) **U.S. Cl.** **530/350; 530/350; 530/300;
435/7.1; 424/192.1; 514/2; 514/12; 514/21**

(58) **Field of Search** **530/350, 300;
435/7.1; 424/192.1; 514/2, 12, 21**

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(57) **ABSTRACT**

A hybrid protein contains a protein that binds to a receptor
of mastocytes and basophils and is endocytosed by them. The
protein can be IgE; IgE fragment; IgE Fc fragment; antibody
against IgE receptor of mastocytes and basophils; fragment
of the antibody against the IgE receptor of mastocytes and
basophils; antibody against mastocyte specific potassium
channel; and mast cell degranulating peptide. The hybrid
protein also contains a protease cleaving proteins of the
secretion process of the mastocytes and basophils so as to
inhibit the secretion process without killing the mastocytes
and basophils. The protease can be light chain *Clostridium*
botulinum toxin; proteolytically active fragment of the light
chain of a *Clostridium botulinum* toxin containing an amino
acid sequence His-Xaa-Xaa-Xaa-His-Xaa-Xaa-His wherein
Xaa is an amino acid; light chain of the tetanus toxin;
proteolytically active fragment of the light chain of the
tetanus toxin containing His-Asp-Leu-Ile-His-Val-Leu-His;
IgA protease of *Neisseria gonorrhoeae*; and proteolytic
domain of the IgA protease of *Neisseria gonorrhoeae*.

11 Claims, No Drawings